phenotype can also include, but is not limited to, a biochemical activity, such as enzymatic, transcriptional, ion channeling, receptor function, structural function, and the like. Such activity could be the activity of the activated protein or an activity induced by the activated protein. A phenotype would also include a morphological event, such as membrane ruffling, and other cellular events, such as transformation or apoptosis, induced by expression of the activated gene.

Applicants point out that their detailed response to the arguments in the Office Action are often directed to the case in which the "phenotype" that is the target of drug screening is a phenotype induced by the activated gene product. This is because the Office Action focuses on this aspect.

Applicants reiterate, however, that a "phenotype" also includes expression of the activated gene itself: i.e., activated RNA and protein from the activated gene. Therefore, Applicants' arguments apply also to the effect of a drug on expression or activity of the product of the activated gene (i.e., RNA or protein).

In summary, when activation of a gene produces a detectable phenotype in a cell, including RNA and protein from the activated gene, that phenotype provides a target by which a compound can be tested for its effect on the phenotype.

Validated/Unvalidated Activated Gene

In one embodiment of the invention, a validated target (i.e., disease correlation is known) is activated. In this embodiment, the methods apply to testing a compound for its ability to affect a phenotype induced by expression of the target or interact with (e.g.,

bind to) the target. Since a disease correlation is known, identified compounds can be tested for efficacy and safety in established disease models.

However, the methods can also be practiced when an unvalidated target is activated. Compounds that affect a phenotype induced by expression of the target or interact with the target, provide candidate compounds for further testing, such as in cellular assays, biochemical assays or in animal disease models. Furthermore, discovering such a compound using the claimed methods provides a step towards validating the target. Identified compounds can then be tested for drug activity in disease models.

<u>Characterized/Less-Characterized Activated Gene</u>

In one embodiment of the invention, a characterized gene is activated. In this embodiment, the methods apply to testing a compound for its ability affect a phenotype induced by expression of the target or interact with the target. One example of a characterized target is a protein with an established biochemical activity, such as a protease activity or ligand binding activity. Using the characterized biochemical activity of the gene product, compounds that modulate that activity can be identified. Thus, expression of characterized genes is useful in the present methods.

However, the methods can also be practiced when a less characterized target is activated. In this embodiment, the methods apply to testing a compound for its ability to affect the phenotype induced by expression of the target or interact with the target. An example of this is the activation of an uncharacterized gene, the expression of which

produces a phenotype in the cell. Even if the biochemical activity of the gene or the mechanism of phenotype formation is not known, the artisan can still screen for compounds that modulate the phenotype. Moreover, the methods can also be practiced when the gene is uncharacterized and induces no detectable phenotype. In this case, the detectable phenotype is expression or activity of the gene product itself. Identified compounds can then be tested in cellular and animal models of disease. Thus, less-characterized genes can be used with the present methods to screen for drugs.

Identity of Activated Gene Established Prior to Drug Screening/Identity of Activated Gene Not Established Prior to Drug Screening

In one embodiment of the invention, the identity of the activated gene is known to the artisan prior to drug screening. As disclosed in the specification, the identity of activated genes can be determined by rtPCR, ELISA, FACS, and other methods known in the art. Thus, the artisan can identify and isolate individual clones expressing a particular gene of interest, and use these clones to identify compounds that affect a phenotype induced by expression of the gene or interact with the gene product.

In another embodiment of the invention, drug screening can be carried out where the identity of the activated gene is not established by the artisan prior to drug screening. In this embodiment, expression of the unidentified activated gene creates a detectable phenotype in the cell or cell supernatant. As one example, if the artisan desired to screen for drugs affecting protease function, cells could be screened with drug candidates in the presence of a protease substrate, e.g., a fluorogenic substrate. Cells containing an

activated protease will cleave the substrate and protease activity will be detected. Accordingly, without determining the identity of the activated gene, it is still possible to screen for compounds that modulate protease activity. Such compounds can then be tested for activity in cellular and animal models. Thus, the artisan can identify compounds that affect a phenotype induced by expression of the gene or interact with the gene product, even when the identity of the gene is not known prior to drug screening.

All Classes of Genes are Activated

As will be evidenced in Applicants' response below, the claimed methods have been established to activate virtually any gene in the genome. Therefore, the Applicants' claimed methods provide a means to test compounds against any of the above types of targets, i.e., validated/unvalidated; highly-characterized/less well-characterized. Further, since virtually any gene can be activated, then any of the corresponding phenotypes can be used for finding compounds that affect any of those phenotypes. Wherever an assay exists for a phenotype, an activated cell expressing that phenotype can be used to identify compounds that affect that phenotype. Such compounds provide, at the very least, primary compounds for further drug development.

Moreover, drug screening can be carried out whether the activated gene has been identified or not prior to screening. In this case, cells having a non-parental phenotype are screened for compounds that affect that phenotype.

Drug Discovery with Recombinant Cells

As a final introduction to the topic, Applicants point out that recombinant DNA technology has allowed the expression of genes, the creation of phenotypes, and consequently the screening for drug candidates using cloned cDNA and genomic DNA fragments. Such cloned fragments produce RNA and/or protein as a screenable phenotype or the RNA and/or protein induce a phenotype that is screenable. Such technology has allowed drug discovery using recombinant genes producing proteins that fall into each of the categories discussed above. The fact that Applicants' targets are expressed from an endogenous sequence in a cell does not present experimental problems in addition to those routinely encountered using recombinant DNA technology, which is commonly practiced in the art.

III. The Rejection

The Rejection Under 35 U.S.C. § 112, First Paragraph.

On page 2 of the Office Action claims 58-61 have been rejected under 35 U.S.C. § 112, first paragraph, on the grounds that these claims are not enabled. Applicants respectfully traverse the rejection.

On pages 2-3 of the Office Action the Examiner discusses the factors generally assessed in determining whether an invention is enabled. The Examiner then concludes "the specification is not enabling for the claimed invention because the specification does not provide sufficient guidance, evidence or exemplification so that an artisan of skill would have been able to make and use the invention as claimed… without undue

experimentation." Applicants respectfully disagree with the conclusion for the reasons introduced above and set forth herein below.

With respect to specific arguments on which the rejection is grounded, the Examiner explains on page 3 as follows:

> ...there is no evidence that all the claimed endogenous genes would have been activated by the claimed methods and would have yielded increased production of proteins from endogenous genes listed. On page 135 of the specification, lines 9-30, continued on page 136, lines 1-3, disclose the results of an experiment of activating expression of transmembrane protein. The specification discloses that in one screening, of 8 isolated activated genes 4 encoded known integral membrane protein genes, whereas 6 encoded novel genes... In yet another example, the specification discloses that of 11 genes isolated, one had sequence homologous to a partially sequenced integral membrane protein gene, whereas 9 were novel genes, for which nothing is known.

First, Applicants point out that the Examiner refers to "endogenous genes listed." However, the claims are not directed to specific genes. As written, claim 59 encompasses the following steps: (1) integrating the vector into the genome to activate expression of an endogenous gene, (2) culturing the cells to allow expression, (3) exposing the cell to a test compound, and (4) ascertaining whether exposure to the test

compound affects a cellular phenotype induced by the gene product or ascertaining whether the test compound interacts with the gene product. Applicants respectfully assert that, based on the Applicants' specification and a general knowledge in the art, the person of ordinary skill in the art would have been able to practice each of these steps using routine experimentation.

With respect to (1), the Examiner takes the position that it would have required an undue burden of experimentation to activate various genes. The Examiner does not provide a rationale for the position other than that the exemplary material does not provide evidence that a wide variety of endogenous genes would have been activated.

Applicants point out that this argument was set forth by the Examiner in co-pending priority U.S. application No. 09/276,820, in the Office Action dated February 1, 2000. In that application, the Examiner concluded that the probability of activating genes other than those exemplified was "small to none", because none of the listed claimed genes was explicitly shown to be activated in the exemplary experiments. Essentially, the Examiner argued there, as appears to be the case here, that the experiments in the specification do not provide evidence that a broad range of genes can be activated using the disclosed vectors and procedures.

In response to that Office Action, the Applicants showed why the experiments in the specification were sufficient to show that a broad range of genes was activated. The Examiner is directed to pages 10-13 of the Response, appended hereto, and also to the appended Declaration by Dr. John Harrington. Dr. Harrington explains why the results described in the specification provide clear evidence that it is reasonably predictable that

a broad range of genes would be activated. He also provides additional data showing how the method can be used to activate virtually any gene in the genome.

With respect to (2), Applicants assert that culturing a cell to allow expression of an activated gene was routine in the art at the time the application was filed and the Examiner has not presented evidence to the contrary.

With respect to (3), the Applicants assert that treating or contacting a cell with a test compound prior to screening for the effect of the compound on gene expression was also routine in the art at the time that the application was filed and the Examiner has not presented evidence to the contrary.

With respect to (4), the Examiner appears to take the position that unless the target was highly characterized and known in advance of screening, an undue burden of experimentation would have been required to discover a useful compound. Applicants disagree for the reasons provided below and introduced above.

The Examiner relies on Caporale (Proc. Nat'l. Acad. Sci. U.S.A. 92:75-82 (1995)) as follows: "Caporale… reviewed some aspects of drug discovery and stated, 'A key step in the process of *selecting a molecular target* for a drug discovery program involves a demonstration that altering the activity of the proposed target should affect the disease." Office Action, page 4, italics added.

Applicants point out that the cited text is directed to *selecting a molecular target for a drug* and not to discovering a drug. Applicants' claims are directed to drug screening once a target or phenotype has been selected by the artisan. Since the claims at issue relate to drug screening and not target selection, the cited text does not apply.

On page 4 of the Office Action, Caporale is again cited. This text describes drug optimization procedures. The problem discussed in this text is non-specific receptor subtype binding. The solution proffered is to select one subtype as a target and to counter-screen the drug against non-target subtypes to find a drug that is specific for the subtype target. This counter-screen provides *drug optimization*, a refinement in the drug discovery sequence. However, the Examiner relies on this text to support the position that the activated gene must be known and characterized prior to drug screening before one "could proceed to the step of compound testing". Applicants respectfully disagree for the reasons introduced above and that follow.

First, Applicants point out that the drug discovery process is not limited to immediate selection of a perfect drug with respect to safety and efficacy. Drug discovery also involves "compound testing" for selecting candidate drugs. These may turn out to have varying degrees of efficacy (for example, target specificity) or safety (degree of toxicity caused, for example, by non-specific cellular uptake or non-specific molecular interaction). But even if a drug is not completely effective or has undesired side effects, this is not evidence that drug discovery using the claimed methods would have required an undue burden of experimentation.

Applicants point out that the claimed methods are simply directed to discovering a compound that interacts with an activated gene product or affects a cellular phenotype caused by activation of the gene. The claims do not (and need not) provide limitations directed to the optimum safety or efficacy of the drug discovered.

In the drug screening process, drug candidates must be initially discovered before these can be subject to further testing for clinical application. Furthermore, in the case of pathological conditions in which no drug is yet available, regulatory standards, such as "clinical efficacy", can be relaxed to provide some treatment even though side-effects can be severe and efficacy not high. Thus, a drug can be discovered by using the claimed methods that is somewhat effective or produces side effects. This issue is not one of patentability but is one of FDA regulation.

On page 4 of the Office Action the Examiner further discusses the position that drug discovery involves undue experimentation unless there is extensive target characterization of the protein and knowledge of the protein's identity prior to drug screening. Specifically, he raises the following issue:

> In the instant case, when the expression of an unknown gene is activated and there is some change in the phenotype of the cell, in the absence of any clues as to what disease such a phenotype or activated gene is related to how would a drug or compound be selected for determining whether it affected said phenotype.

The Examiner then discusses "the main issue" as follows:

> Therefore, the main issue is: if the artisan did not know what gene has been activated how would the artisan test for the activity of a drug because for what activity is the artisan going to look for, in

other words how would an artisan know that a given phenotype is because of one activated gene or more than one activated gene or whether the phenotype produced is the result of the expression of one gene alone that in turn may be activating multiple genes, for example, in a signal transduction pathway.

As summarized in the Introduction above, wherever gene activation creates a detectable phenotype, the cell can be screened for a drug that affects that phenotype. The expressed gene need not be known and no phenotype or disease correlation need be known. A compound that affects a phenotype is *per se* useful as a candidate for secondary testing.

As discussed, the "activity" that the artisan "looks for" is a phenotype. The phenotype could be expression of the gene product or could be a biochemical activity, a morphological event, or cellular event resulting from expression of the activated gene. Any activation phenotype would serve as a target for modulation by the candidate drug. Thus, the activated gene need not be characterized or even identified in advance of drug screening.

Applicants point out that the Examiner is focused on the instances in which the activated gene is not identified or not well-characterized (e.g., can only be classified based on, for example, a functional sequence motif) in advance of drug screening. This, however, represents only a subset of genes that are activated using the claimed methods. Genes that are activated include virtually every gene in a cellular genome (based on

experiments described in the appended Declaration and results described in Applicants' specification). Thus, in many cases the gene that is activated is one that has been highly characterized.

Applicants also point out that the specification describes methods for determining the identity of activated genes in pools of cells and in isolated clones. Disclosed methods include rtPCR, ELISA, FACS, and other methods known in the art. Applicants further point out that each of these techniques has been used successfully by the Applicants to determine the identity of activated genes in isolated clones and pools of clones (see Declaration by John Harrington. Thus, if desired, it is possible to determine the identity of the activated gene prior to drug screening.

However, Applicants reiterate that the claimed method can be used equally well to identify drug candidates whether or not the activated gene is characterized or identified. Drug discovery based on phenotypic screening and/or gene product interaction can be practiced without an undue burden of experimentation. The process is as follows: The vector is integrated into a cell; the cell develops a specific phenotype as a result of gene activation; the phenotype is not present in the parent cell, for example, cellular transformation; without knowing the nature or identity of the gene activated, this cell is used to screen for compounds that modulate the phenotype. Such screening would lead to the discovery of drugs that can then serve as candidates for further testing.

The Examiner raises the issue of drug interaction with a gene product other than the activated gene product. Applicants point out that drug discovery using the claimed methods can be used both when the activated gene is the target of the drug and when the

activated gene is not the target of the drug. The drug may, in fact, act on genes that are activated or inhibited in response to the gene product of the activated gene. In this case the activated gene itself would not be the target. Nevertheless the *activation* results in a *phenotype* that can be used to discover drugs that affect it.

Accordingly, the artisan would not in fact have to "first characterize the gene activated, its protein's functions, its localization in a cell and what parts of the protein are important for its function before it could proceed to the step of compound testing" (Office Action, page 4).

The Examiner also relies on Czerwinski *et al.* (Proc. Nat'l. Acad. Sci. U.S.A. 95:11520-11525 (1998)) for providing some of the "requirements of screening a successful drug". Again, by using the term "successful", Applicants believe that the Examiner is limiting the claims to those drugs that provide minimal side-effects and maximum efficacy. However, as noted above, a useful drug need not be maximally safe or effective.

The passage cited by the Examiner shows a typical problem encountered in drug development for a maximally safe and effective drug. The specific problem cited in the reference (page 5 of the Office Action) is one of non-specific entry of a drug into a cell. Applicants point out that this problem is specific to one mechanism of drug action (i.e. targeting drugs for cellular uptake into tumor cells by interaction with cell surface receptors). This problem, therefore, does not apply to other drugs, such as receptor agonists, receptor antagonists, and enzyme inhibitors, or to other mechanisms of drug action. Thus, the cited example does not apply in most instances of drug development.

Furthermore, this type of problem may occur for this one drug strategy. However, this does not mean that an undue burden of experimentation generally would have been required to select a candidate drug using Applicants' methods.

Applicants point out that in recent decades, recombinant DNA technology has allowed the artisan to express proteins, including novel proteins, create phenotypes, including phenotypes produced by novel proteins, and screen for drug candidates using cloned cDNA and genomic DNA fragments. The present invention provides the artisan with an ability to activate and express genes on a genome-wide basis without cloning the target gene of interest. This allows the artisan to create cells expressing target genes and displaying phenotypes of interest. Once a cell is selected for screening, the screening process used in the present method can be the same as methods that are commonly used to identify compounds using recombinant cells.

Many sources of compounds suitable for such screening were known in the art. Examples include, but are not limited to, natural compound libraries, synthetic compound libraries, including combinatorial chemistry libraries, existing drug compounds, and other known and novel compounds. The screening process depends on the target or phenotype of interest and the assay to be used. A large number of assays was known in the art. These include, but are not limited to, receptor binding, ion flux, transcription reporter assays, enzyme assays, and the like. Any or all of these would have been known to be useful in the present methods at the time the application was filed. Thus, the artisan, armed with the present methods and any one of the many known screening assays and compound libraries, could have routinely screened for drug candidates.

In summary, useful drugs can be discovered using Applicants' claimed methods without knowing the nature or identity of the gene that is activated. This is done by assessing the effect of a candidate compound on a phenotype that is produced in a cell following introduction of an activation construct. The nature of the activated gene or gene product is immaterial to the issue of phenotypic screening. The cell with the phenotype induced by introduction of the activation vector is screened for drugs, using routine and normal experimentation, which drugs have an effect on the phenotype. The activated gene may or may not be the target. What is crucial is that the phenotype is affected by the drug.

On page 5 of the Office Action, the Examiner then raises the issue of "what to screen for" if the protein is not full length. As the Examiner recognizes, in some embodiments, the protein might not be full length.

Applicants point out that many truncated proteins retain one or more of the activities present in the full-length protein. Thus, any activated protein with a detectable activity (such as ligand binding or enzymatic activity) can be screened for compounds that bind to the protein, enhance its activity, or inhibit its activity.

In addition, even a partial coding sequence with no biochemical activity provides a phenotype useful for drug screening. For example, such proteins may contain functional sites, or protein motifs (e.g. zinc fingers, binding pockets, alpha helices, beta sheets, beta turns, reverse turns, or other structures) that allow interaction of a drug candidate with the protein. In these instances, drug candidates obtained from the screening of the truncated protein may be found to interact with or modulate the activity

of the full-length protein. Furthermore, the truncated proteins, with or without biochemical activity, can be used to generate antibodies. Antibodies to human proteins have been shown to be effective drugs in many diseases.

Applicants also point out that truncated proteins are also capable of generating detectable phenotypes when expressed in cells. Indeed, both dominant negative and dominant positive phenotypes have been observed by over-expressing truncated proteins in a cell. Thus, truncated proteins are also useful in the present method since they are capable of generating phenotypes for drug screening. One power of the claimed method, accordingly, is that those cells having a detectable phenotype will be detected. Since integration is non-targeted and genome-wide, cells will be produced in which a given gene is not full-length but of sufficient length to produce a detectable phenotype.

The rejection is further grounded on the position that an undue burden of experimentation would have been required to incubate the isolated protein or concentrated conditioned medium with the drug unless the nature of the protein encoded was known. The Examiner argues as follows:

> Without knowing the nature of the protein encoded how would an artisan have known what kind of compounds to test. For example, without knowing the hydrophobic or hydrophilic nature of the protein, how would an artisan determine the assay conditions for the interaction of the protein or the drug.

See page 6 of the Office Action

Applicants point out that even when screening highly characterized targets, it is often difficult or impossible to predict which compounds will be active drugs. Accordingly, methods used in the art involve screening of compound libraries, often containing thousands to hundreds of thousands of diverse compounds, to identify those compounds that interact with the target, modulate its activity, or alter a phenotype induced by expression of the target. Less characterized and uncharacterized targets would be screened using the same methods. For example, anti-cancer screens often involve screening tumor cells (expressing a large number of uncharacterized, and indeed unknown targets) with compound libraries to identify compounds that inhibit proliferation, cause tumor cell death, or otherwise affect the tumor cell. Thus, the degree of characterization does not unduly influence the selection of compounds for screening.

With respect to assay conditions, the artisan would use conditions under which the activity or phenotype is observed. Under these conditions, the targets or cells would be exposed to test compounds to identify compounds that interact with the target, modulate its activity, or alter a phenotype induced by expression of the target. Thus, by using an identified activity (e.g., kinase, protease, etc.) or phenotype (e.g., growth in soft agar) to carry out the screening process, the artisan could simply use the existing assay conditions to carry out the drug screening process.

The rejection is further based on arguments found on page 7 of the Office Action. In the first full paragraph the Examiner speculates that a protein expressed at low levels in concentrated medium might lose its function or activity when concentrated. He speculates that "cell growth medium may have inhibitors of the said protein or other

enzymes such as proteases that may degrade the candidate protein". He then concludes that "without knowing the characteristics of the protein", the specification provides no guidance about "how to deal with such a problem".

First, Applicants point out that the above argument applies only to claims 60 and 61. Claims 58 and 59 do not involve protein purification or concentration.

Second, Applicants point out that the Examiner has provided no evidence to suggest that an unreasonable number of embodiments would be subject to this alleged limitation.

Third, Applicants point out that a number of established protein concentration and purification techniques are disclosed in the specification. These include, but are not limited to, ion exchange chromatography, hydrophobic chromatography, affinity and immunoaffinity chromatography, ammonium sulfate precipitation, and ultrafiltration. All of these approaches have been used with great success for many, if not most proteins tested. Many of these methods can be employed without any knowledge of the hydrophobic or hydrophilic nature of the protein of interest. For example, Applicants point out that specific vectors and methods are described for expressing epitope tagged proteins from activated genes, thereby allowing rapid isolation and purification. Epitope tagging has been used to isolate a very large and growing number of proteins with great success. Again, this approach can be employed without specific knowledge of the protein's identity or physico/chemical properties. Thus, this approach can be used along with the approaches described above to isolate the target of interest.

Fourth, Applicants point out that target purification would remove any inhibitors present in the media. Applicants also point out that methods for culturing cells in serum-free media are disclosed in the specification. In this embodiment, "inhibitors present in the growth media" would be minimal or non-existent. Thus, the presence of inhibitors, even if present, can be addressed using methods disclosed in the application.

Fifth, Applicants point out that even in instances in which the activity of a protein is reduced during purification, the activity is usually not lost entirely. Sufficient residual activity often remains that allows successful compound testing.

Applicants have disclosed methods for protein purification and concentration in the specification. These methods have been used successfully for both characterized and uncharacterized proteins. Thus, the claimed methods directed to isolation and concentration of the protein prior to screening can be practiced without an undue burden of experimentation.

Summary Conclusion

Drug discovery can be practiced without an undue burden of experimentation using Applicants' claimed methods as long as there is a detectable phenotype produced by activation. The phenotype could be expression of the activated gene product *per se* or a phenotype induced by the activated gene product. Cells (or products thereof) expressing the phenotype are exposed to candidate compounds to assess the ability of the compound to interact with the activated gene product or affect a phenotype induced by the activated product.

The activated gene product could be, but need not, be well-characterized. As long as a screenable phenotype is produced upon activation, a compound can be routinely assayed for its effect on the phenotype.

The activated gene product could be, but need not be, known prior to drug screening. As long as a screenable phenotype is produced upon activation, a compound can be routinely assayed for its effect on the phenotype.

Drug screening using recombinant cells was routine in the art at the time that the application was filed. Such screening utilized novel as well as well-characterized genes. The claimed methods present no experimental obstacles in addition to those normally encountered in drug discovery using recombinant cells.

In view of the above evidence and discussion, Applicants submit that the grounds for rejections have been addressed and the rejection overcome. Reconsideration and withdrawal of the rejection is therefore respectfully requested.

Applicants believe that the present application is now in condition for allowance. If the Examiner believes, for any reason, that personal communication will expedite prosecution of this application, the Examiner is invited to telephone the undersigned at the number provided. Prompt and favorable consideration of the foregoing response is respectfully requested.

It is not believed that extensions of time or fees for net addition of claims are required, beyond those that may otherwise be provided for in documents accompanying this paper. However, in the event that additional extensions of time are necessary to

allow consideration of this paper, such extensions are hereby petitioned under 37 CFR § 1.136(a), and any fee required therefore (including fees for net addition of claims) is hereby authorized to be charged to Deposit Account No. **50-0622**.

28 August 2000

Respectfully submitted,

\# 43,172

Anne Brown
Registration No. 36,463

Shanks & Herbert
TransPotomac Plaza
1033 N. Fairfax Street #306
Alexandria, VA 22314
Phone: (703) 386-6134
Fax: (703) 683-9875

Certificate of Mailing

I hereby certify that this correspondence is being deposited with the United States Postal Service with sufficient postage as first class mail in an envelope addressed to:

Assistant Commissioner for Patents
Washington, DC 20231

on 28 Aug 2000
(Date)

Typed or printed name of person signing this certificate.
Suzannah K. Sundby

Signature: